United States Environmental Protection Agency

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title:	Assessment of disinfected drinking water and disinfection by-products for developmental toxicity in rats
LAPR Number:	20-07-003
Principal Investigator	Exemption 6
Author of this Document:	Exemption 6 /RTP/USEPA/US
Date Originated:	06/23/2017
LAPR Expiration Date:	07/31/2020
Agenda Date:	07/12/2017
Date Approved:	07/24/2017
Date Closed:	

APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6 Exemption 6 Exemption 6 /RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	07/24/2017	DMR	
	Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US by Exemption 6 RTP/USEPA/US	07/24/2017	DMR	

Administrative Information

1. Project Title (no abbreviations, include species):
Assessment of disinfected drinking water and disinfection by-products for developmental toxicity in rats

Is this a continuing study with a previously approved LAPR?
Yes

Please provide the previous LAPR# 17-06-002

2. Programatic Information

a. What Program does this LAPR support? Please provide the Research Program, Project, Task Number and Title.
Safe and Sustainable Water Resources (SSWR) Task 2.2.D: Integrated Assessment and Reduction of Contaminant Risks

b. What is the Quality Assurance Project Plan (QAPP) covering this project?
IRP: NHEERL-RTP/TAD/ETB/Exempti Exempti Exempti/2014-001-r0

3. EPA Principal Investigator/Responsible Employee:

Principal Investigator Exemption 6	***Phone Number*** Exemption 6 ***Lotus Notes Address*** Exemption 6 Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US	***Division*** TAD ***Branch*** ETB	***Mail Drop*** MD

4. Alternate Contact:

Alternate Contact Exemption 6	***Phone Number*** Exemption 6 ***Lotus Notes Address*** Exemption 6 Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US	***Division*** TAD ***Branch*** ETB	***Mail Drop*** MD

SECTION A - Description of Project

1. Explain the study objective(s) in <u>non-technical language</u> such that it is understandable by non-scientific persons. <u>Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research.</u> If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.

Although disinfection of public water supplies has been a major success in decreasing disease, the disinfectant reacts with materials in the source water to produce hundreds of disinfection by-products (DBPs). Chlorinated water has been found to contain more than 600 compounds, and many DBPs remain unidentified - the known DBPs account for only approximately one-half of the mass of halogenated organic matter. Although chlorination is the disinfection method used by most US water utilities, an alternative method, chloramination is becoming increasingly common because it forms smaller amounts of the EPA-regulated DBPs. However, chloramination (i.e., disinfecting with chloramine, the result of combining chlorine plus ammonia) produces other DBPs, many of which with little, if any, toxicological data available.

Epidemiological studies, as well as toxicological studies in laboratory animals, have raised concerns regarding possible adverse health effects of DBPs. Of particular note, DBPs have been associated with heart malformations in rodents as well as in epidemiology studies. However, it should be noted that many of the DBPs remain unidentified, the vast majority of the known DBPs have not been investigated toxicologically, and that little is known about the potential interactions among the DBPs. Therefore, we are using a "whole-mixture" approach to evaluate the toxicity of the complex mixtures produced by disinfection in order to better characterize the potential health risks of exposure to disinfected water. This "whole-mixture" includes unidentified contaminants along with the well-characterized DBPs, and also includes potential interactions of chemicals in mixture.

In our previous work (Exemption 6 Exemption 6 et al., 2013, Environ. Sci. & Technol. 47:10653), we conducted a multigenerational toxicity study to assess the reproductive effects of water that was concentrated 136 fold and then chlorinated. The 136x water was palatable for the rats and, reassuringly, the study was largely negative, particularly for prenatal mortality and pup weight. However, there were slight, but significant, delays in puberty and reductions in sperm counts. The Office of Water deemed this study to be very valuable, and has asked us to conduct a similar study to compare the developmental toxicities of chlorinated water and chloraminated water. Unlike our previous study, we plan to use a different approach to produce whole mixtures. Here, we have obtained freeze-dried natural organic matter (NOM) from source water. The freeze-dried NOM will be reconstituted (with purified laboratory water) to produce water at various concentrations. The reconstituted water will be split into three "streams", one stream to be chlorinated, one chloraminated, and one raw (neither chlorinated nor chloraminated) to serve as a control. These streams, along with an additional control group of purified water, will allow us to conduct developmental toxicity evaluations of chlorinated and chloraminated waters. Thus, this research will provide further insights into the potential developmental toxicity of real-world mixtures and aid in EPA's risk assessments of chlorinated and chloraminated drinking waters, consumed by millions of people worldwide.

Prior to conducting full developmental toxicity assessments of chlorinated and chloraminated waters in F344 rats, we plan to conduct a preliminary palatability study with nonpregnant females. In our previous LAPR (17-06-002), a palatability study confirmed that rats readily drank chlorinated NOM water that was concentrated 60 fold, but chemistry analyses revealed that levels of brominated and iodinated DBPs were much lower than levels typically found nationally. Brominated and iodinated DBPs are generally more potent that chlorinated DBPs and may play an important role in causing adverse health effects in communities with relatively high levels of these DBPs. Therefore, we wish to spike the concentrated NOM mixture with bromide and iodide to raise the levels of brominated and iodinated DBPs to be consistent with 90th-percentile levels found nationally, multiplied by the target concentration factor (60x). We wish to conduct a palatability test with the increased brominated/iodinated DBP levels to ensure that the rats will drink these waters before conducting the main developmental toxicity study.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

In vivo testing is an essential part of assessing developmental toxicity hazard in that the intact live animal is the only test system available that incorporates the complex maternal-embryonic interactions of development.

b. Justify the species requested:

Rats have been used extensively in this field because of their size, ease of care, fecundity, and large historical database. Our work will gain insights from, and add to, the large historical database of developmental toxicity research in this species.

3. How was it determined that this study is not unnecessary duplication?

A search of PubMed confirms that we are the only laboratory evaluating developmental toxicity of mixtures of disinfection by-products in rodents, and is not unnecessary duplication. Search terms: developmental toxicity, rats, mixtures, disinfection by-products, drinking water.

SECTION B - In Vivo Procedures

1. Briefly describe the experimental design. Include descriptions of the age, weight and sex of the animals. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.

Experiment 1. Palatability study.

To assess whether or not the rats will drink the water preparations at these higher concentrations, we plan to conduct a palatability study with four treatment groups (five rats per group).

Non-pregnant female rats will be provided water from one of the four treatments for up to 10 days.

Freeze-dried NOM will be reconstituted with purified water (see Section D) to a volume to provide water that is 60-fold more concentrated than its source water. Prior to disinfection, the reconstituted NOM will be spiked with potassium bromide (KBr) and potassium iodide (KI) to raise the levels of brominated and iodinated DBPs. One group will receive chlorinated concentrate, one group will receive chloraminated concentrate, and a control group will receive raw (non-disinfected) concentrate. An additional control group will receive purified water.

Control - purified water
Control - raw concentrated water (60x)
Chlorinated concentrate (60x)
Chloraminated concentrate (60x)

Experiment 2. Developmental Toxicity Study

Timed-pregnant F344 rats, obtained by in-house breeding, will be provided water from one of the four treatments from gestation day (GD) 6 through 16. This exposure period encompasses the period of embryonic organogenesis.

Treatment groups are as described for the palatability study; however, if 60x water is not palatable, the concentrations will be reduced accordingly.
Control - purified water
Control - raw concentrated water (60x)
Chlorinated concentrate (60x)
Chloraminated concentrate (60x)

Each exposure group will have two cohorts (euthanasia time points):

Euthanized on GD 16 (10 timed-pregnant females per group)
Dams will be euthanized at the end of the 10-day exposure period for blood and other tissue collection to determine DBP blood levels and DBP effects on tissues.
- CO2 euthanasia for terminal blood draw from abdominal aorta
- Tissues (e.g., liver, kidney, thyroid) will be weighed and fixed for histological examination
- Uterine contents will be examined for implantation sites, resorption sites, conceptus viability, fetal weight

Euthanized on GD 21 (26 timed-pregnant females per group)
Dams will be euthanized at term, allowing examination of full-term fetuses

- Gravid (pregnant) uterus is weighed
- Uterine contents examined for implantation sites, resorption sites, live fetuses
- Live fetuses weighed, sexed, examined, and euthanized for further examination of heart, viscera, skeleton

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.

Experiment 1 (palatability)
Based on our data and experience with drinking water studies, five animals per group should be adequate to identify any notable palatability issues.
4 groups x 5 rats/group = 20 rats

Experiment 2 (Developmental toxicity study)
Euthanasia on GD 16
Based on previous experience with the blood and histological endpoints, we expect that six to eight pregnant animals per group will be sufficient to discern differences between groups. Based on historical pregnancy rates, we request 10 timed-pregnant females per group.
4 groups x 10 timed-pregnant females/group = 40 timed-pregnant females

Euthanasia on GD 21
Regulatory guidelines for developmental toxicity studies recommend approximately 20 dams (i.e., with confirmed pregnancy) per treatment group. Based on historical pregnancy rates, we expect that 26 timed-pregnant females per group will give us the recommended number of confirmed pregnancies.
4 groups x 26 timed-pregnant females/group = 104 timed-pregnant females

Total timed-pregnant females needed: 144 (40 euthanized GD 16, 104 euthanized GD 21)

Timed-pregnant (i.e., mated) females will be obtained by in-house breeding.
We plan to purchase 90 males to be used for multiple breedings, up to approximately 20 months of age. Based on previous experience with in-house breeding, we anticipate that up to about one-half of these males may be unreliable breeders. Unreliable breeders (i.e., males that do not mate with estrous females) may be culled (transferred to another LAPR or euthanized). Thus, after initial breeding sessions to obtain timed-pregnant females for other LAPRs, we anticipate having approximately 45 reliable breeders to provide an appropriate number of mated (timed-pregnant) females each day during breeding.

Based on previous experience using reliable male breeders, we expect about 75% of the females to successfully mate in 1 week of breeding. (Note that because of variations in estrous cyclicity, even with reliable male breeders, some females are not receptive during the breeding period and will not mate.) Thus, we request 192 females to obtain 144 mated (timed-pregnant) females.
Purchase 192 females to obtain target of 144 mated (timed-pregnant) females

Overall: 20 rats (Experiment 1) + 90 male breeders + 192 females (Experiment 2) = 302 total

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	302	0
D) Potential pain/distress relieved by appropriate measures:		
E) Unrelieved pain/distress:		

4. Does this LAPR include any of the following:

☐ Restraint (>15 Minutes) ☐ Survival surgery
☐ Food and/or water restriction (>6 Hours) ☐ Non-survival surgery

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):

Exposures will be by drinking water, provided continuously, ad libitum.
Palatability study: 10-day exposure
Developmental toxicity study: 10-day exposure (GD 6-16)

In the palatability study, NOM concentrations will be equivalent to source water concentrated 60x. If this initial study raises concerns regarding palatability, the concentration will be reduced accordingly in the subsequent developmental toxicity studiy.

b. Survival Blood Collections (method, volume, frequency):

c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):

d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:

e. Breeding for experimental purposes (e.g. length of pairing, number of generations):

General approach:
Two females will be placed in each male's cage. The following morning, each of the cohabiting females will undergo vaginal lavage and the smears will be examined microscopically for vaginal sperm (i.e., evidence of mating). Sperm-positive females will be removed from the male, assigned to the study, and housed with another mated female (two mated females per cage). Females that did not mate will remain with the male. Near the end of the workday, additional non-pregnant females will be placed with available males and the process will repeat each day. Generally, this procedure will be conducted 4 days per week (e.g., sperm positive on Tuesday through Friday) to coordinate study events (e.g., euthanasia dates) with work schedules. At the end of the week, any non-mated females will be removed to prevent unwanted breeding over the weekend, and the process will be continued the next week as needed.

Vaginal lavage:
Vaginal lavage will be conducted each morning after cohabitation with males. Presence of a copulatory plug or sperm-positive vaginal smear will be considered evidence of mating.

As the breeding phase of the study progresses, and a limited number of females becomes available for breeding, vaginal lavage may be conducted prior to cohabitation in order to identify receptive females.

Vaginal lavage will be conducted by laboratory staff or Animal Care Staff (a Technical Service Request will be submitted). Using a glass medical dropper or disposable plastic pipette, water will be inserted into the vaginal opening, aspirated back into the dropper/pipette, and placed on a glass slide for microscopic examination for vaginal cytology and the presence of spermatozoa.

Housing:
Upon receipt, juvenile males will be housed two per cage until approximately 1 week prior to their first breeding. At that time, the males will be housed individually and remain one per cage (except when breeding) until termination. Cages will be provided with heat-treated pine shavings for bedding, and enviro-dri (or similar) for enrichment.

Non-pregnant females will be housed two per cage. During breeding, one female will be placed in a male's cage until mated for up to five consecutive days. Females that have not mated at the end of the week's breeding will be removed from the males' cages and re-housed two per cage with other non-pregnant females. Sperm-positive (pregnant) females will be pair housed.

Animal ages:
Males and non-pregnant females may be purchased as young as 3 weeks of age. Males will be used for breeding between 2 and approximately 20 months of age. Females will be used for breeding usually at 9-15 weeks of age (approximately 130-200g).

Females that have been cohabited with males but did not show evidence of mating will be observed for indications of pregnancy. Females observed to be pregnant (i.e., an untimed-pregnancy, and therefore cannot be assigned to the study) will be transferred to an appropriate LAPR or euthanized.

f. Describe how animals will be identified and monitored. Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom:

Animals will be identified by ear tag.

Prior to and after breeding periods, all animals will be monitored weekly by research staff (listed in Section E). During the breeding periods, all animals will be monitored daily on weekdays by research staff. If animals show signs of physical injury or declining body condition, the animal will be euthanized or we will otherwise follow AV recommendations.

During treatment periods, animals will be monitored daily on weekdays by research staff.

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):

b. Blood Collection (Provide a description of the procedure including method, volume, and frequency if appropriate. Indicate if the procedure is survival or terminal. Include preparatory methods, descriptions of incisions, etc.):

c. Testing methods:

d. Restrictions placed on the animals' basic needs (e.g., food and/or water restriction, light cycles, temperature). Provide details regarding the length of restriction. Describe the method(s) for assessing the health and well-being of the animals during restriction. (Amount of food or fluid earned during testing and amount freely given must be recorded and assessed to assure proper nutrition.):

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):

f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:

7. Surgical Category D and E procedures. Indicate if the surgery is survival or terminal. Describe each surgical procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:

b. Anesthetic regimen (Drugs, dosages, volume, route of administration and delivery schedule). The use of paralytic or neuromuscular blocking agents w/o anesthesia is prohibited:

c. Postoperative care (thermal support, special feeding, responsible personnel, removal of sutures/staples, frequency and duration of monitoring including weekend and holiday care):

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):

e. Will any animal be subject to more than one surgical procedure over the course of its lifetime, either here at NHEERL or elsewhere?

◯ Yes ◯ No

f. Identify any surgical procedures performed at other institutions or by vendors:

8. Humane interventions (for treatments/procedures in all categories).

a. What resultant effects, if any, do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.

If drinking water treatments result in markedly reduced water consumption, leading to dehydration and/or deteriorating body condition, treatment may be discontinued (i.e., we will provide tap water or purified water to the animals) to allow rehydration.

We do not anticipate any maternal toxicity. However, if dams show symptoms of physical injury, severe toxicity, marked dehydration, dystocia, or deteriorating body condition we will euthanize or otherwise follow AV recommendations.

b. State the criteria for determining temporary or permanent removal of animals from the study. Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.

If animals show symptoms of physical injury, severe toxicity, marked dehydration, dystocia, or deteriorating body condition we will euthanize or otherwise follow AV recommendations.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study: 302

b. Animals to be transferred from another LAPR:

LAPR Number that is the source of this transfer:

c. Animals to be transferred from another source:

d. Offspring produced onsite (used for data collection and/or weaned):

e. TOTAL NUMBER of animals for duration of the LAPR 302

2. Species (limited to one per LAPR): Rat

3. Strain: F344

Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)

none

4. Sources of animals:

Envigo or other

5. Provide room numbers where various procedures will be performed on animals:

Exemption 6
Exemption 6
Exemption 6

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.

no *Room Numbers:*

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)

none

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.

none

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

We will submit a technical service request for vaginal lavage of females when cohabited with males.

10. Housing and Enrichment.

The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.

Heat-treated pine shavings will be used as bedding.

Upon receipt, all animals will be pair housed.

Animals used in the palatability study will be singly housed in order to record individual water consumption. Enviro-dri (or similar) will be provided as enrichment.

Males will be singly housed approximately 1 week prior to their first breeding; enviro-dri (or similar) will be provided as enrichment.

Enviro-dri or similar is acceptable for use with the palatability study, the male breeders, and unmated females. However, because of concerns of endocrine disruptors, it will not be used for timed-pregnant females; note, the timed-pregnant females will be pair housed.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used.
Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.

Freeze-dried natural organic matter (NOM), obtained from the International Humic Substances Society, reconstituted with milli-Q® highly purified water and spiked with KBr and KI.
The reconstituted water will be divided into three streams:
chlorinated, chloraminated, or undisinfected, and provided as drinking water.

Maximum dose: 60x the concentration of source water (based on dissolved organic carbon).
KBr and KI will be added to achieve brominated and iodinated DBP levels approximating the average of the five US states with the highest levels (160 ug/L and 16 ug/L of bromide and iodide, respectively) multiplied by the multiplication factor (60x).

LD50 data are unavailable.

2. Describe compounds to be administered to animals.

a. Are all substances pharmaceutical grade? If not, provide a scientific justification for the use of non pharmaceutical grade compounds.

Pharmaceutical grade KBr and KI will be used.
Pharmaceutical grade NOM is not available.

b. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in the LAPR. Provide information to assure that such material is pathogen free. Indicate what safety precautions are necessary for handling the material.

none

c. Provide a statement regarding any safety precautions necessary for handling any of these materials.

Gloves, laboratory coat, and safety glasses will be used when preparing or handling these materials.

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:

Hint: *The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).*

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6	Principal Investigator	Study design; body weights, water consumption, clinical observations, cervical dislocation, Category C procedures	Completed NHEERL-required training. >30 years experience. Proficient in cervical dislocation, including rats >200 g.
Exemption 6	Technical Staff	body weights, water consumption, clinical observations, Category C procedures	Completed all NHEERL-required training. Mentored by Exemption 6.
Exemption 6	Associate Principal Investigator	Study design, Category C procedures.	Completed NHEERL-required training. >30 years experience.

Exemption 6	Technical Staff	Body weights, water consumption, clinical observation. Category C procedures.	Completed NHEERL-required training. >20 years experience.
Exemption 6	Technical Staff	Body weights, water consumption, clinical observation. Category C procedures.	Completed NHEERL-required training. >20 years experience.
RTP-NHEERL	Tech Support	Category C Procedures	All NHEERL required training is complete.

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

1. Estimated number of breeding pairs and liveborn per year

2. Breeding protocols and recordkeeping

3. Methods for monitoring genetic stability

4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR

SECTION G - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

Palatability study: animals will be euthanized when the palatability study is completed.

Male breeders: euthanized by approximately 20 months of age.

Timed-pregnant females: GD 16 or GD 21.

Unmated females: Approximately 17 weeks of age.

2. Describe the euthanasia techniques:

Method(s): Cervical dislocation or CO2 asphyxiation
Agent(s): CO2
Dose (mg/kg): to effect
Volume:
Route: inhalation

Source(s) of information used to select the above agents/methods:

2013 AVMA Guidelines on Euthanasia., Personal experience

3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).

Cervical dislocation of rats >200g: The 2013 AVMA Guidelines for the Euthanasia of Animals recommends cervical dislocation as a method of euthanasia for rats weighing <200g when performed by individuals with a demonstrated

high degree of technical proficiency. It also states that the large muscle mass in the cervical region of heavy rats makes manual cervical dislocation physically more difficult. The Guideline's 200-g weight limit is flawed for two important reasons: 1) The additional weight acquired during pregnancy or lactation has little, if any, influence on the muscle mass of the neck. (E.g., our F344 rats typically weigh 200-250 g during late pregnancy, but their nongravid weights are <180g). 2) The technique for performing cervical dislocation described by the AVMA Guidelines is appropriate for mice, but it is an inferior technique for rats. Rather than using the thumb and index finger, the preferred technique involves placing the index and middle fingers on either side of the animal's neck (from the dorsal aspect with the palm facing rostrally). Unlike the Guideline's method, this method IS appropriate for heavier animals and is NOT physically more difficult. The Principal Investigator of this project has >30 years experience performing this technique on nongravid rats weighing >350g and pregnant or lactating rats weighing >500g.

4. Describe how death is to be confirmed.
Prolonged absence of breathing

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.
Euthanized as above
Euthanized by Animal Care Contractor
Transferred to another study
The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?
● Yes ○ No

SECTION I - Assurances

1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.
2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.
3. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
4. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.
5. The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.
6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.
7. I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.
8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date
Exemption 6 Exemption 6	07/05/2017

Submitted: 07/05/2017

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	***Approval Date***	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	07/05/2017 by Exemption 6 Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	Exemption 6 ***Lotus Notes Address*** Exemption 6 Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	TAD ***Branch*** ETB	MD ***Submitted to Branch Chief for Approval*** 07/05/2017 11:35 AM

ATTACHMENTS

20-07-003 PI resp1.pdf

Actions

First Update notification sent: 05/29/2018
Second Update notification sent:
First 2nd Annual notification sent:

Second 2nd Annual notification sent:

1st Expiration notification sent:
2nd Expiration notification sent:

History Log: